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I. Biology of Legionellosis

Etiologic Agent

*Legionellae* are Gram-negative bacilli. More than 34 species of *Legionella* with a number of distinguishable serogroups have been identified. *L. pneumophila* is responsible for greater than ninety percent of infections. Eighteen serogroups of *L. pneumophila* are currently recognized. The majority of the disease reported in the United States is caused by serogroup one. *Legionellae* thrive in warm, aquatic environments and are relatively resistant to the effects of chlorine and heat.

*Legionellae* are generally spread through the air by aerosolized water which is then inhaled or microaspirated. Contaminated aerosols come from devices such as cooling towers, showers, and faucets. Infection has also occurred by contamination of surgical wounds with potable water. It is not transmitted from person to person.

Clinical Features

Legionnaires’ disease (legionellosis) is a bacterial infection that was first identified following a 1976 outbreak of pneumonia at an American Legion Convention in Philadelphia. Persons with Legionnaires’ disease may present early in the illness with nonspecific symptoms, so it can be difficult to diagnose. Signs of the disease can include: a high fever, chills, and a cough. Some people also suffer from muscle aches and headaches. Twenty to forty percent of cases exhibit gastrointestinal symptoms. The clinical presentation of Legionnaires’ disease is not generally clinically distinguishable from other causes of community-associated pneumonia. This can lead to cases of legionellosis being mis-diagnosed. The incubation period for Legionnaires’ disease is 2-10 days with an average of 5-6 days.

Chest X-rays are needed to diagnose the pneumonia caused by the bacteria. However, it is not possible to diagnose legionellosis based on radiographic evidence only. Chest radiographs of patients with legionellosis are clinically indistinguishable from those of patients with pneumococcal pneumonia or mycoplasma pneumonia, which are common causes of community-associated pneumonia. Because of this, laboratory testing is required to identify cases of legionellosis. These tests can include cultures or PCR tests on sputum, blood, or lung tissues but the most common diagnostic test is the urine antigen test.

A milder infection caused by the same type of Legionella bacteria is called Pontiac Fever. Symptoms are similar to Legionnaires’ disease with fever, headache, and muscle aches, however, there is no pneumonia. The incubation period for Pontiac Fever is 5-66 hours, most often 24-48 hours. Symptoms of Pontiac Fever go away on their own in two to five days without treatment and without causing further problems.

Each year between 8,000 and 18,000 people are hospitalized with Legionnaires’ disease in the United States. Although Legionnaires’ disease can occur at any time of year, the greatest number of illnesses are usually found in the summer and early fall.

II. Epidemiology of Legionellosis
National Data

People most at risk of getting sick from infection with *Legionella* bacteria are older people (usually 65 years of age or older), as well as people who are smokers, or those who have a chronic lung disease (like emphysema).

People who have weak immune systems from diseases like cancer, diabetes, or kidney failure are also more likely to get sick when exposed to *Legionella* bacteria. People who take drugs to suppress (weaken) the immune system (like after a transplant operation or chemotherapy) are also at higher risk.

Florida Data

The distribution of legionellosis in Florida does not differ from the national trends. The majority of cases in Florida occur in those 65 and over with a peak incidence in those 75-84. When looking at seasonality, the majority of cases occur in August to October.

![Figure 2. Legionellosis Cases by Month of Onset, Florida, 2007](image)

![Figure 3. Legionellosis Incidence Rate by Age Group, Florida, 2007](image)

III. Case Management
Case Definition

The Florida Department of Health case definition for legionellosis:

Clinical description:
Legionellosis is associated with two clinically and epidemiologically distinct illnesses:
Legionnaires disease, which is characterized by fever, myalgia, cough, and clinical or radiographic pneumonia, and Pontiac fever, a milder illness without pneumonia.

Laboratory criteria for diagnosis:
Confirmatory:
• Isolation of any Legionella organism from respiratory secretions, lung tissue, pleural fluid, or other normally sterile fluid,
OR
• Detection of Legionella pneumophila serogroup 1 antigen in urine using validated reagents,
OR
• Fourfold or greater rise in specific serum antibody titer to Legionella pneumophila serogroup 1 using validated reagents.
Supportive:
• Fourfold or greater rise in antibody titer to specific species or serogroups of Legionella other than L. pneumophila serogroup 1 (e.g., L. micdadei, L. pneumophila serogroup 6);
OR
• Fourfold or greater rise in antibody titer to multiple species of Legionella using pooled antigen and validated reagents;
OR
• Detection of specific Legionella antigen or staining of the organism in respiratory secretions, lung tissue, or pleural fluid by direct fluorescent antibody (DFA) staining, immunohistochemistry (IHC), or other similar method, using validated reagents;
OR
• Detection of Legionella species by a validated nucleic acid assay.

Case classification
Confirmed: A clinically compatible case with confirmatory laboratory evidence.
Suspect: A clinically compatible case with supportive laboratory evidence.

Comment
The previously used category of “probable case,” which was based on a single IFA titer, lacks specificity for surveillance and is no longer used.

Travel-associated: A case that has a history of spending at least one night away from home, either in the same country of residence or abroad, in the two weeks before onset of illness. Indicate if the case is travel-associated in the case notes.

Case Investigation
(See also Environmental Assessment Section)
Confirmed and suspected cases of legionellosis should initially be interviewed using the following case report form: http://www.cdc.gov/legionella/files/legionella_case_report.pdf. If the patient is unable to be interviewed then the investigator should try to interview a spouse, parent or guardian, relative, or someone that can act as a proxy and is knowledgeable about the
patient’s history. In addition to demographic and laboratory information the investigator should inquire about any medical or dental visits, the patient’s occupation, and any travel that occurred in the two weeks before onset.

Symptoms of Legionnaires’ disease include: rapid onset of high fever 39°- 40.5°C (102°-105°F), chills, nonproductive progressing to productive cough, anorexia, malaise, headache, and sometimes abdominal pain and diarrhea. A chest x-ray will show evidence of pneumonia. The incubation period is 2-10 days (most often 5-6 days, but may be as long as 14 days). Pontiac fever manifestations are similar but is not associated with pneumonia. Patients with Pontiac fever have a self-limiting illness that will resolve in 2-5 days.

When investigating reported or suspected cases of legionellosis, epidemiologic risk factors should be assessed and noted in the investigation. Risk factors may include:

- Recent travel with overnight stay away from home
- Whirlpool spa exposure
- Renal or hepatic failure
- Diabetes
- Immune system disorders
- Malignant cancers
- COPD
- Congestive heart failure
- Smoking
- Males
- > 65 years of age

Please fax the completed case report form to the Bureau of Epidemiology at (850) 922-9299. If the case is travel-associated please inform the Bureau of Epidemiology and other department partners by posting to the EpiCom forum, also notify your regional environmental epidemiologist and the case reviewer at the Bureau of Epidemiology.

Physicians and other clinicians requesting consultation regarding treatment and management of patients with Legionnaire’s disease should refer to the most recent guidelines from the Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) on community-acquired pneumonia:

http://www.journals.uchicago.edu/doi/pdf/10.1086/511159?cookieSet=1

**Single Confirmed Case Associated with a Health Care Facility or Other Facility**

Single cases that spent their entire incubation period in the facility should trigger a full epidemiological and environmental investigation.

**Investigation of a Cluster of Two or More Confirmed Cases of Legionellosis Linked to the Same Facility**

A confirmed cluster or outbreak of legionellosis in which two or more individuals have a person, place, time epidemiological link to a facility triggers a more intensive environmental assessment of the identified facility. It is extremely important to utilize a team approach when performing a facility assessment. A team may consist of a pool/spa specialist, an indoor air specialist, an industrial hygienist, and an epidemiologist. It is also important to have the regulatory agency present during the assessment, although it is not necessary to accompany them as they inspect
different items. Contact your regional environmental epidemiologist for assistance and guidance.

An epidemiologic investigation should be initiated within 24 hours of notification of information of two or more suspected legionellosis cases that are associated to a common place within an appropriate time period. Coordination between the epidemiologic investigation and the environmental health assessment should begin as early as possible. The objectives of the epi investigation are to identify any other potential cases (active case finding) and to determine the most likely source of an outbreak of legionellosis by establishing associations between ill individuals and possible exposures. These associations can direct environmental health control measures, especially in the absence of environmental culture data.

Please see Appendix A for the Legionellosis Investigation Worksheet developed for Florida.

Additional sample questionnaires that may assist in case investigation:

IV. Environmental Assessment

Investigation

There are two response levels that can be initiated: one for a sporadic case of legionellosis and a more intensive response to a single facility linked case or an outbreak or cluster of legionellosis.

Investigation of Confirmed Sporadic Case of Legionellosis

A sporadic case of legionellosis is a single confirmed case of legionellosis in which an individual has identified a number of facilities in which they resided or other possible exposure (e.g. spa, fountain) during the incubation period of the disease. A sporadic case of legionellosis does not trigger a full scale environmental assessment of the multiple facilities/exposures unless there is recent, prior history of legionellosis linked to the facility/exposure source (see cluster guidance below for these types of incidents), however, the following steps may be taken to raise awareness and prevent future cases of legionellosis: The facility should be notified of the sporadic case of Legionella (without breaching HIPAA or patient confidentiality rules). Sample letters are available at http://www.cdc.gov/legionella/index.htm. The facility’s regulatory agency (in the case of hotels, the Department of Business and Professional Regulation) should be notified of the sporadic case of legionellosis. If the facility has a spa regulated by FDOH, a complaint investigation/inspection can be conducted to ensure compliance with spa regulations. A review of the spa maintenance log is recommended to determine if any breaks in sanitization or maintenance has occurred recently. Obtain a copy of the maintenance log for your records. If the spa chemistry and filtration is in compliance at the time of inspection, the inspector can, as a precautionary measure, recommend the facility follow a spa remediation protocol or if the facility’s spa is out of compliance at the time of inspection the inspector can require the facility to follow spa remediation protocol (see Remediation Issue Section).

Single Confirmed Case Associated with a Health Care Facility or Other Facility or Investigation of a Cluster of Two or More Confirmed Cases of Legionellosis Linked to Exposure
Single cases that spent their entire incubation period in the facility should trigger a full epidemiological and environmental investigation. A confirmed cluster or outbreak of legionellosis in which two or more individuals have a person, place, time epidemiological link to a facility triggers a more intensive environmental assessment of the identified facility. It is extremely important to utilize a team approach when performing a facility assessment. A team may consist of a pool/spa specialist, an indoor air specialist, an industrial hygienist, and an epidemiologist. It is also important to have the regulatory agency present during the assessment, although it is not necessary to accompany them as they inspect different items. Contact your regional environmental epidemiologist for assistance and guidance.

Environmental Assessment of Facilities with Suspected/Confirmed Cases of Legionellosis

An environmental health assessment should be initiated within 24 hours of notification of information of two or more suspected legionellosis cases that are associated to a common place within an appropriate time period. Coordination and receipt of appropriate sample collection materials may take a day or two, but initial steps to gather information and coordinate efforts can begin in the interim period. Typically implicated places are group residences, meeting rooms, dormitories, hotels, hospitals, and other mass gathering facilities. The objective of the environmental health assessment is to determine the most likely source of an outbreak of legionellosis or prevent legionellosis from occurring by lowering a facility’s risk factors for *Legionella* propagation and growth.

It is important to understand that reported cases of legionellosis may not be confirmed for 3-6 weeks after onset of illness. This will factor into the types of samples collected and assessment methods. It is also important to conduct such investigations in a strictly confidential manner, as with many other infectious diseases, in order to minimize unwarranted alarm and adverse economic impact.

Extensive written records of any relevant facts and observations are crucial to the assessment. The assessment should focus on possible amplification sites associated with mechanisms capable of aerosol generation. The known epidemiological data of the cases should also be obtained and considered prior to the onsite assessment and subsequent to any environmental findings or sampling results. Transmission of the infectious organism most likely involves respirable droplets of less than 5 microns in size that contain the organism. Droplets of this size are invisible and have the physical properties of gas.

Gather Information

Prior to Arriving On Site:

- Establish a time line of diagnosed illness in relation to possible exposures at home, work, while traveling, or during visits to atypical locations.
- Inquire about any interim remedial measures that may have been taken by the Facility Manager that could impact your sampling plan.
- Identify possible amplification sites that also pose the risk of aerosolizing water droplets.
- Request and review plumbing diagrams of the building if available.
- Request and review water treatment reports for cooling towers if present or available.
- Identify the locations of outside air intakes for HVAC systems in relation to cooling towers or fountains.
- Inquire about, or review “as-built” mechanical plans for, the presence of humidifiers within HVAC systems or stand-alone humidifiers.
Use the information gathered prior to the site inspection to estimate how many samples you may need to collect and of what type. Also determine the necessary types of equipment you will need to safely gain access to the sampling sites. Determine what will be needed to stop the aerosolization of water from potential amplification sites during sample collection. Identify means to prevent unexpected or unauthorized reactivation of pumps, fans, or other mechanical devices that aerosolized water (Lock-Out/Tag-Out). Determine what types of tools you will need to remove shower heads, faucet aerators, or pump access covers, locations where a biofilm often forms and where samples should be taken.

**Environmental Assessment: General Information**

A. Do not conduct random, undirected microbiologic sampling of air, water, and environmental surfaces in suspected facilities.

B. When indicated, conduct microbiologic sampling as part of an epidemiologic investigation or during assessment of hazardous environmental conditions to detect contamination and verify abatement of a hazard.

Environmental sampling, as with all laboratory testing, should not be conducted if there is no plan for interpreting and acting on the results obtained. Linking microorganisms from environmental samples with clinical isolates by molecular epidemiology is crucial whenever it is possible to do so.

**Environmental Assessment for *Legionella* Amplification Sites:**

**Key Factors to aid in identification of possible *Legionella* amplification sites:**

1. Stagnant water and/or plumbing designs and installations that result in stagnation, such as side-arm and dead-leg piping;
2. Warm water temperatures between 20 and 50ºC (68 to 122ºF);
3. Optimal growth is at temperatures between 35 and 45ºC (95 to 113ºF);
4. Bulk water pH in the range of 5.0 to 8.5;
5. Sediment, scale, deposits, biofilm – support not only *Legionella* growth, but also the growth of the very important supporting microbiota for *Legionella*;
6. Microbiota, including algae and many bacteria that supply essential nutrients for growth of *Legionella*;
7. Certain amoebae and other protozoa that harbor *Legionella* as endosymbionts – allowing them to thrive, resist harsh environmental conditions (including biocides), and to amplify.

Many different types of water systems can serve as *Legionella* amplifiers and (aerosol) disseminators, and have been associated with Legionnaires’ disease. They include:

- Domestic Hot Water Systems (tap faucets, showerheads, sprayers),
- Cooling Towers and Evaporative Condensers,
- Spas and Whirlpools (on display or otherwise in use),
- Humidifiers,
- Water fountains,
- Decorative Fountains,
- Supermarket Reservoir Misters,
- Respiratory Therapy Equipment
Legionella Colonization Frequencies: (Hodgson & Casey study, 1998)
- Potable Water Distribution Systems 12.0%
- Hot Water Heaters 7.0%
- Cooling Towers 6.3%

Equipment

Staff conducting environmental assessments should have appropriate equipment including: camera or video equipment, flashlights, thermometers, pool/spa test kit for chlorine, bromine, pH and cyanuric acid, domestic water test kit for chlorine and pH, a quaternary ammonia test kit and small tools to open inspection panels. An indoor air specialist or industrial hygienist may bring other sampling equipment such as smoke tubes or micromanometer, to determine the direction of air flow.

Health and Safety Considerations

Safely conducting an environmental assessment starts with hazard recognition and mitigation. While inadvertent exposure to Legionella pathogens may seem to be the primary risk, the more commonplace hazards of slips and falls, cuts and abrasions on corroded equipment, and electrical shocks are more likely. The following discussion is intended to make the inspector aware of some of the possible hazards they may encounter in the course of collecting samples for Legionella, but does not purport to be all inclusive or comprehensive.

The question of whether or not an inspector collecting water samples for Legionella should wear respiratory protection is frequently asked. In order to protect workers from known or suspected hazards the two primary approaches available in this situation are engineering controls and personal protective equipment (PPE), which includes respiratory protection. Whenever possible, engineering controls to prevent exposure to the hazard are recommended before PPE is used. Since the primary hazard to the person collecting samples is inhalation of aerosolized water, preventing the aerosolization of water while samples are being collected is best way to prevent exposure. The OSHA Technical Manual on Legionnaires Disease concludes that respiratory protection is necessary when a significant risk of exposure to high concentrations of contaminated aerosols exists. By eliminating the aerosols during sampling, the need for respiratory protection is negated.

By turning off or disabling any pumps, fans, or other mechanical means that aerosolizes water, the sample may be collected in the safest manner. This approach solves two hazards at once, first it prevents inadvertent physical hazards from the pump or fan pinching or crushing an appendage, or an electrical shock, and second it stops the aerosolization of water while the samples are being collected. The recommended approach to preventing inadvertent reactivation of the pump or fan is by using appropriate lock-out equipment. If all measures are taken to cease water aerosolization mechanisms during sample collection, then respiratory protection should not be necessary. To avoid aerosolizing water from showers and faucets, the shower head or faucet aerator should always be removed to collect water and to facilitate inspection and sampling of biofilm on the back side of the shower head or faucet aerator.

The inspector should also take measures to avoid slips, trips and falls, electrical hazards, and overhead hazards. Non-slip shoes, goggles, nitrile gloves, and a hard hat may be appropriate when entering a cooling tower to collect samples. Avoid wearing ties or other loose clothing that may become caught on nearby machines or surfaces. Be aware of inadvertent exposures to cleaning or water treatment chemicals often used and stored in and around cooling towers. The
On Site Assessment

Focus on those areas that are biologically plausible for *Legionella pneumophila* to grow, amplify and be aerosolized. During the site inspection, determine if there have been any changes in routine operations. Of particular interest are conditions within the incubation period of 2-10 days, but most likely 5-6 days prior to the onset of symptoms of reported cases. Has there been any construction onsite or any interruption of water supply to the building? Has the facility been under a boil water notice? Has the fire suppression system recently been tested? Remember that extensive written records of any facts and observations which could be relevant should be kept.

**Water sources that frequently provide optimal conditions for growth of the organisms include:**

1. Cooling towers, evaporative condensers, and fluid coolers that use evaporation to reject heat. Consider the following:
   A. Are there any located within 200 yards of air intakes or such proximity to people?
   B. Visible condition.
   C. Is an algaecide or biocide used.
      i. Name of product.
      ii. Active ingredient.
      iii. Concentration as determined by field test.
   D. Water temperature.
   E. Dates tower was operated.
   F. Date tower was last serviced.
   G. Service company information.

2. Domestic hot water systems with water heaters that operate below 60°C (140°F) and deliver water to taps below 50°C (122°F). Consider the following:
   A. Supplier.
   B. Monochloramine.
   C. Free or combined chlorine residual.
   D. Temperature at proximal and distal locations.
   E. Re-circulated.
   F. Backflow prevention.
   G. Water heaters or break tanks.
   H. Dead legs in plumbing designs.
   I. Shower heads-design and location.
   J. Don't bother testing tankless water heaters.

3. Humidifiers and decorative fountains that create water spray and use water at temperatures favorable to growth. Consider the following:
   A. Visible condition.
   B. Is an algaecide or biocide used?
   C. Name of product.
   D. Active ingredient.
   E. Concentration as determined by field test.

inspector should always be conscious of wasps, hornets, bees, and other stinging insects that often build nests near roof top mechanical equipment. As this is Florida and lightning storms can be frequent and unexpected, avoid working on any roof tops when thunder storms threaten.
F. Water Supply.
G. Filtered.

4. Spas and whirlpools. Consider the following:
   A. Visible condition.
   B. Chlorine/bromine concentration, pH, cyanuric acid level.
   C. Type of filters.
   D. Date last backwashed.
   E. Date last drained and scrubbed.
   F. Temperature.
   G. Visible biofilm layer (check skimmer baskets too).
   H. Fill water supply.
   I. Review and obtain copy of maintenance records.
   J. Adequate ventilation.

5. Other sources: stagnant water in fire sprinkler systems and warm water for eye washes and safety showers, dental water lines, which are frequently maintained at temperature above 20°C (68°F) and sometimes as warm as 37°C (98.6°F) for patient comfort. Consider the following:
   A. Date last flushed.
   B. Temperature.

A useful assessment tool which can be modified, depending on the situation, can be located at http://www.cdc.gov/legionella/index.html

For workplace investigation guidelines, see also OSHA Technical Manual Section III, Chapter 7, pg 7 of 40 https://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_7.html


Sampling: See Laboratory Testing Guidelines section for sampling procedures

Remediation: See Remediation Section

V. Laboratory Testing Guidelines

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<thead>
<tr>
<th>Diagnostic tests for legionellosis</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Culture</td>
<td>• Clinical and Environmental Isolates can be compared • Detects all species and serogroups • 100% specific</td>
<td>• Technically difficult • Slow (&gt;5 days to grow) • Sensitivity highly dependent of technical skill • May be affected by antibiotic treatment</td>
</tr>
<tr>
<td>Urine Antigen</td>
<td>• &gt;99% specific • Rapid (same day)</td>
<td>• Only for L. pneumophila serogroup 1 (Lp1) which may account</td>
</tr>
</tbody>
</table>
for up to 80% of cases
- Limited utility when compared to environmental isolates

| Serology | • Not affected by antibiotic treatment
• 70-80% sensitive; > 90% specific |

| DFA      | • Can be performed on pathologic specimens
• 95% specific |

|          | • Must have paired sera
• 5-10% of population has titer ≥1:256
• Single acute phase antibody titers of ≥1:256 do not discriminate between cases of Legionnaires’ disease and community-acquired pneumonia |

• 25-75% sensitive

The Bureau of Public Health Laboratories processes confirmatory tests on patient specimens for serology tests and culture tests.

For serology tests, acute and convalescent sera must be submitted. These samples should be 1-3 mL of serum at onset (acute) and once again 21 days later (convalescent). These samples should be collected in plain, red-topped, vacutainer tubes and submitted at the same time. A positive result is a titer > 1:256, and recent infection is demonstrated by a fourfold rise (IgG) or fall (IgM) in titer between the acute and convalescent sera.

For culture tests, eligible specimens are sputum, bronchial brushings, bronchial washings, lung biopsy, or normally sterile fluid. These should be submitted in sterile screw-capped containers that are taped shut. Results should be available in ~3 days but this can vary based on type of sample submitted and the presence of a back log of tests at the lab.

**Specimen Submission**

All patient specimens should be transported to the laboratory in insulated coolers as protection against extreme heat or cold. Specimens that will reach the laboratory within 24 hours can be shipped at room temperature. Specimens that will not reach the laboratory within 24 hours should be refrigerated until they are shipped and then packaged with a cooling agent. Specimens that reach the laboratory but cannot be processed within 24 hours of collection should be refrigerated until they are processed.

**Specimen Findings Interpretation**

Findings should be interpreted with caution. Several *Legionella* organism serotypes are found in nature. The significance of positive finding is dependent on the pathogenesis of the particular organism, its presence at a high enough level to constitute an infective dose, delivery to lungs via respirable size water droplets and the susceptibility of the persons exposed to disease.
development. The importance of the laboratory findings will probably not be known, and in fact, should not be evaluated without the complete data from the epidemiological investigation.


Environmental Samples

Do not conduct random, undirected microbiologic sampling of air, water, or environmental surfaces. When indicated, conduct sampling as part of an epidemiologic investigation or during assessment of hazardous environmental conditions to detect contamination and to verify effective remediation of the source. The CDC and Florida Department of Health advocate sampling after Legionnaires’ disease has been diagnosed in a single case with only one exposure to a facility or when two or more persons (suspected or confirmed) in order to locate the source of Legionella and take remedial action. They do not encourage sampling in the absence of suspected or confirmed Legionnaires’ disease.

Sampling

Two types of media should be sampled when evaluating sources for Legionella amplification;
1. Water for free floating bacteria, and
2. Biofilm for amplification sites

Collection of water samples for pathogen isolation from drinking water or surface waters should be performed by DOH County Health Departments due to the risk of possible infection to the sample collector. Gloves and possibly goggles (see page 11, Equipment) should be used when handling potentially infectious materials.

An environmental sampling protocol addressing selection of the appropriate sites to sample has been previously published (Barbaree et. al, 1987; see references). Whenever possible, collect 1 (one) liter of water from potable water sources. Larger volumes of water (1 to 10 liters) are occasionally needed to detect Legionellae in water that has very low concentrations of these bacteria such as municipal water supplies. If a liter cannot be collected from a sample source, a smaller volume is acceptable, but may result in a higher limit of detection.

Water samples should be collected in a sterile 1-liter wide-mouth screw cap polypropylene plastic bottle. If the water source has been recently treated with chlorine, add 0.5 ml of 0.1N sodium thiosulfate to each 1 liter sample to neutralize the disinfectant. Sampling containers obtained from the Bureau of Public Health Laboratories, and most private labs, will already contain the appropriate amount of sodium thiosulfate as a tablet inside of the container or dried on the inside of the container, and can be supplied to the CHD within 24 hours.

Swabs of faucet aerators and shower heads should be taken before water samples from these sites. The sample should be taken from the back side of the aerator or shower head after it has
been removed. Sterile polyester swabs with wooden shafts work well for this purpose. The swabs should be placed in a sterile screw-cap container and submerged in 3-5 ml of water taken at the same time to prevent drying during transport.

The presence of biofilm within a water source greatly increases the likelihood that *Legionella* is present. Collecting samples from biofilm in sediments, on screens, and in scale improves the likelihood of identifying the amplification sites for *Legionella*.

Document the aerosolization potential for water sources. Ensure they are inactivated while you are sampling. Wait 15 to 20 minutes after the aerosolized water has been stopped before entering the area. This is to allow the aerosols to settle out of the air and reduce the risk of exposure to the investigator.

**Sample Collection Equipment**

Include in your field kit the following items:

- Thermometer
- pH paper or meter (optional)
- 1 Liter polypropylene plastic bottles for potable water samples collection (with sodium thiosulfate)
- 100-250 mL sterilized polypropylene plastic bottles for cooling tower water collection
- Sterile polyester swabs for biofilm samples
- Paper towels for cleaning and drying exterior of bottles
- Vinyl tape to seal bottle tops
- Indelible marker (water proof) to record sample ID on bottles and swab tubes
- Tools to remove faucet aerators and shower heads
- Lock-out/tag-out equipment to prevent accidental reactivation of pumps or fans (optional)

Having sufficient materials to modify your assessment in the field when conditions turn out to be different from what you expected allows you to more effectively identify potential sources without having to return to the site later.

**Environmental Sample Analysis**

Samples may be analyzed by the laboratory by either culture of viable organisms or polymerase chain reaction (PCR), or both. If PCR is chosen, one option is Quantitative PCR (QPCR), which provides a quantitative estimate of bacterial concentration, but does not rely upon the bacteria being viable.

The Bureau Public Health of Laboratories can analyze samples for *Legionella* using culture based methods, but does not perform QPCR for *Legionella*. Private laboratories may also analyze samples using either culture based or QPCR methods. A list of accredited environmental microbiology labs can be found at [https://wwwn.cdc.gov/elite/Public/MemberList.aspx](https://wwwn.cdc.gov/elite/Public/MemberList.aspx)

<table>
<thead>
<tr>
<th>Advantages of culture-based analysis</th>
<th>Disadvantages of culture-based analysis</th>
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<tbody>
<tr>
<td>Quantitative results (cfu/mL) allows interpretation of data as it relates to source strength.</td>
<td>Application of biocides to the water source may prevent detection.</td>
</tr>
<tr>
<td>Serotyping of bacteria allows comparison with the organism serotype found in the infected</td>
<td>Analysis takes 7 to 10 days.</td>
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<tr>
<td>Sodium thiosulfate must be added to samples to prevent chlorine in water from killing</td>
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patients bacteria during shipping.

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<tr>
<th>Advantages of PCR analysis</th>
<th>Disadvantages of PCR analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis can be performed in 1 to 2 days. Quantitative results if QPCR method is used (cell equivalents/mL). Treating the water with biocides prior to sampling does not prevent detection of <em>Legionella</em>. Costs the same or less than culture.</td>
<td>Identification to species level can be accomplished, but serotyping of organisms is not possible.</td>
</tr>
</tbody>
</table>

**Sample Submission**

All environmental samples should be transported to the laboratory in insulated coolers as protection against extreme heat or cold. Samples that will reach the laboratory within 24 hrs can be shipped at room temperature. Samples that will not reach the laboratory within 24 hrs should be refrigerated until they are shipped and then packaged with a cooling agent. Samples that reach the laboratory but cannot be processed within 24 hrs of collection should be refrigerated upon receipt and until they are processed.

**Sample Findings Interpretation**

Findings should be interpreted with caution. Several *Legionellae* organism serotypes are found in nature. The significance of positive finding is dependent on the pathogenesis of the particular organism, its presence at a high enough level to constitute an infective dose, delivery to lungs via respirable size water droplets and the susceptibility of the persons exposed to disease development. The importance of the laboratory findings will probably not be known, and in fact, should not be evaluated without the complete data from the epidemiological investigation.

**Report Findings and Make Recommendations**

The levels requiring action vary for the source of exposure based on the assumption that some routes of exposure result in a greater dose to the lung. For this reason, humidifiers and similar devices such as misters and evaporative condensers which produce an aerosol mist that can be directly inhaled should be controlled to lower levels. Remember that these numbers are only guidelines, and the goal is zero detectable *Legionella* in a water source. Levels of *Legionella* equal to or greater than the values in the table constitute a need for action, as described below.

- **Action 1**: Prompt cleaning and/or biocide treatment of the system.
- **Action 2**: Immediate cleaning and/or biocide treatment. Take prompt steps to prevent employee exposure.

**Colony forming units (cfu) of Legionella per milliliter**

<table>
<thead>
<tr>
<th>Action</th>
<th>Cooling Tower</th>
<th>Domestic Water</th>
<th>Humidifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1,000</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

The role of the County Health Department investigator is to identify the likely source of exposure leading to confirmed cases of Legionnaire’s disease, and to assist the facility owner with remediation of the contamination by providing guidance and recommendations. In some cases the facility owner may hire an environmental consultant or industrial hygienist to perform the
initial and follow up assessments. In this situation, the CHD investigator may provide technical oversight and verify that the methodologies and procedures used to sample potential sources and to remediate confirmed amplification sites are appropriate. However, technical review of protocols for environmental assessment or remediation does not relieve the Contractor, Consultant or Building Owner of their responsibilities for proper and safe conduct of remediation efforts. CHDs may elect to consult with DOH regional environmental epidemiologists, indoor air specialists, toxicologists and industrial hygienists.

CHD Investigators should refer to the OSHA Technical Manual section on Legionnaires Disease for further technical guidance on conducting environmental assessments and remediation efforts, available at: https://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_7.html


VI. Remediation

Documents within this section include fact sheets related to the remediation of *Legionella* contamination in several different environments including:

A. Cooling Towers, HVAC Systems, Individual Air Conditioning Units
B. Domestic Hot Water Systems Emergency Management and Best Practices
C. Decorative Fountains and Waterfalls
D. Emergency Management for *Legionella* in Heated Whirlpool Spas

These fact sheets should be used as references within the Department of Health but are also designed to facilitate information distribution among facilities that have experienced problems associated with *Legionella* contamination.
Cooling Towers, HVAC Systems, Individual Air Condition Units

Background Information:

For cooling tower remediation guidance, please consult: Legionellosis – Guideline: Best Practices for Control of Legionella, Cooling Technology Institute (see references and appendices)

HVAC Systems are not normally amplification sites for Legionellae. The organism cannot survive without water, and a properly operated, well-maintained HVAC system is unlikely to be a source of problems. However, an HVAC system that is not properly maintained can disseminate contaminated water aerosols containing Legionella from either an outside source or an internal humidifier. The sources of these aerosols are either external or internal.

External sources release contaminated aerosolized water that is drawn into a system’s fresh-air intake. Mist discharged from cooling towers, evaporative condensers, and fluid coolers can be ingested by the HVAC fresh air intake.

Internal sources may provide contaminated aerosolized water that is then disseminated by the air-distribution system. Contaminated water can leak from pipes into HVAC ducts, where it can be aerosolized and distributed by the system. Potential sources of contaminated water include: domestic water systems, fire-sprinklers, humidifiers and refrigeration condensers.

Remediation:
To minimize risk of Legionella contamination of HVAC systems:
1. Minimize the use of water reservoirs, sumps and pans.
2. Provide a way to drain water sumps when not in use.
3. Provide a “bleed” for water sumps so dissolved solids do not form sediments in the sump.
4. Slope and drain sumps from the bottom so that all the water can drain out and allow the pan to dry.
5. Locate HVAC fresh-air intakes so they do not draw in mist from a cooling tower, evaporative condenser or fluid cooler into the system.
6. Operate and maintain all HVAC equipment as originally designed.

**HVAC systems that serve rooms in which a whirlpool spa is located should be maintained and physically cleaned on a weekly to monthly basis. **

Legionella has not been found to grow in car or window air-conditioner units.

For more information regarding Legionella and HVAC systems please reference the OSHA Technical Manual Chapter 7.

Contact Information:
County ________________________________

Contact Person __________________________

Phone Number ____________________________

You may also reach the Florida Department of Health, Bureau of Epidemiology at 850-245-4401.
Domestic Hot Water Systems: Emergency Management and Best Practices

Background Information:

The following protocol for minimization of *Legionella* in Potable and Emergency Water Systems has been adapted from the ASHRAE Standard 12-2000, Minimizing the Risk of Legionellosis Associated with Building Water Systems and is recommended in cases where Legionnaires' Disease is known or suspected and may be associated with the potable water system. In no way however, should these recommendations be interpreted to guarantee the absence of *Legionella* bacteria or any other particular pathogen, and consequently that these measures will prevent illness (e.g. legionellosis). Nevertheless, these measures can be effective in fostering the safety of the potable water system. This is accomplished through the destruction of free-swimming bacteria, including *Legionella*, and indirectly by eliminating conditions that favor *Legionella* amplification (multiplication), i.e. the elimination of biofilms and amoebae and other protozoa that feed on biofilms and which serve as *Legionella* hosts.

Where decontamination of hot water systems is necessary the following control measures should be taken:

**Thermal Pasteurization:**

1. The hot water tank temperature should be raised to 160 to 170 °F (71-77 °C) for 24 hours.
2. After 24 hours, and while maintaining the water temperature each tap/outlet should be progressively flushed for a minimum time of 5 minutes as recommended by the Center for Disease Control Hospital Infection Control Practices Advisory Committee. Longer flushing times (20 minutes) may be necessary as recommended in the OSHA Technical Manual Chapter 7.
3. The number of outlets that can be flushed simultaneously will depend on the capacity of the water heater and the flow capability of the system.
4. Extreme caution should be used to avoid serious burns from the high water temperatures used in pasteurization.

For systems where thermal shock treatment is not possible, shock chlorination may provide an alternative treatment option.

**Shock Chlorination:**

1. Chlorine should be added to achieve a free chlorine residual of at least 2mg/L throughout the system.
2. This may require chlorination of the water heater or tank to levels of 20 to 50mg/L.
3. The pH of the water should be maintained between 7.0 - 8.0.
4. Each outlet should be flushed until the odor of chlorine is detected.
5. The chlorine should remain in the system for a minimum of 2 hours (not to exceed 24 hours)
6. Thoroughly flush the system.

**Maintenance After Decontamination:**

Note: Once decontamination is complete, recolonization is likely to occur unless the proper temperatures are maintained, continuous supplemental chlorination is continued, or alternative approaches such as the use of silver/copper ionization, ozonation or ultraviolet radiation are employed.
For maintenance of the domestic water system and to minimize growth of Legionella, domestic hot water should be stored at a minimum of 140 °F (60 °C) and delivered at a minimum of 122° F (50°C) to all outlets. However, great care should be taken to avoid scalding through the use of preset thermostatic mixing valves. In buildings that cannot be retrofitted with mixing valves periodically increasing the temperature to 150 °F or chlorination followed by flushing should be considered.

Systems should be inspected annually to ensure that thermostats are functioning properly. Hot water tanks should be drained periodically to remove scale and sediment, cleaned with a chlorine solution if possible and then thoroughly flushed. In high risk facilities such as health care facilities or nursing homes monthly removal of shower heads and tap aerators to clean out sediment and scale and to clean them in a chlorine bleach solution is recommended. Domestic hot-water recirculation pumps should run continuously.

The domestic water system in buildings includes all piping, hot water heaters, storage tanks, faucets, nozzles and other distribution outlets. Factors associated with the plumbing system that may influence the growth of Legionella include:

A. Chlorine concentration,
B. Temperature,
C. Plumbing system design and plumbing materials.

For more information regarding Legionella and domestic water systems please reference the ASHRAE Guideline 12-200 and the OSHA Technical Manual Chapter 7. Another good reference is https://awt.org/IndustryResources/Legionella03.pdf

Contact Information:

County ________________________________
Contacts Person _________________________
Phone Number__________________________

You may also reach the Florida Department of Health, Bureau of Epidemiology at 850-245-4401.
Decorative Fountains and Waterfalls

Definition:
A decorative fountain or waterfall is a man-made system in which water is either sprayed into the air or cascades over a steep media (rocks) and returns to a man-made pool. Several multiple-case outbreaks of legionellosis have been associated with decorative fountains in public buildings including hotels. Decorative fountains can produce droplets less than 5 micron size and the risk for legionellosis increases as the rate of aerosol production increases.

Background Information:
Decorative fountains and waterfalls are sometimes operated intermittently with on-time scheduled only during certain time periods. This may allow for water stagnation during off-times and may encourage greater microbial growth. *Legionella* needs high temperature ranges for proliferation, outdoor fountains and pools in hotter climates and indoor fountains and pools subject to sources of heat may allow the water to become an amplifier. Heat sources may come from the pump/filter systems themselves, underwater lighting or intermittent operation may allow temperature increases in limited parts of the system.

Best Practices:
To limit *Legionella* proliferation or amplification in decorative fountains and waterfalls several items require consideration:

System design:
- Drains or sumps should be situated at the lowest level of the pool, with no other local low points that are not served by drains or sumps.
- Provision for maintenance should be considered in the design stage.
- Access to pump(s) and filter(s) should be provided. Stagnant areas or areas that are difficult to clean should be avoided

Maintenance:
- Regular cleaning is recommended
- Use of filters should be considered, systems with small water volumes may be drained and refilled with fresh water weekly in lieu of filtering

Water Treatment:
A biocide for use in decorative fountains may be used.

For more information regarding *Legionella* in decorative fountains and waterfalls please review the ASHRAE Guideline 12-2000 (see references).

Contact Information:

County ________________________________
Contact Person __________________________
Phone Number___________________________

You may also reach the Florida Department of Health, Bureau of Epidemiology at 850-245-4401.
Emergency Management for Legionella in Heated Whirlpool Spas

Background Information:

The following protocol for minimization of Legionella in whirlpool spas has been adapted from the Cooling Technology Institute, Legionellosis Guideline: Best Practices for Control of Legionella for emergency decontamination of cooling towers and evaporative condensers and is recommended where there are known or suspected cases of Legionnaires disease that may be associated with the whirlpool spa. In no way however, should these recommendations be interpreted to guarantee the absence of Legionella bacteria or any other particular pathogen, and consequently that these measures will prevent illness (e.g. legionellosis). Nevertheless, these measures can be effective in fostering the safety of spa pools. This is accomplished through the destruction of free-swimming bacteria, including Legionella, and indirectly by eliminating conditions that favor Legionella amplification (multiplication), i.e. the elimination of biofilms and amoebae and other protozoa that feed on biofilms and which serve as Legionella hosts (Cooling Technology Institute, February 2000).

Emergency Disinfection Procedures: (Continue to operate the re-circulating pumps)

1. Add a biocide (chlorine or bromine) sufficient to achieve 25ppm to 50ppm free halogen residual.
2. Maintain a 10ppm free halogen (chlorine or bromine) residual for 24 hours. Add more biocide as needed to maintain the 10ppm residual.
3. Monitor system pH. Acid may be added in order to achieve and maintain a pH of less than 8.0 (for chlorine based biocides) or 8.5 (for bromine-based biocides)
4. Drain system (remove& discard filters)
5. Fill spa and repeat steps 1 through 4.
6. Physically scrub all accessible spa surfaces (including filter tanks, skimmer baskets and weirs) to remove any microbial biofilm.
7. Fill system, install new filter elements, maintain a 10ppm halogen residual for 1 hour, and return spa to service.

A monitoring program to detect the recurrence of Legionella may be warranted.

Maintenance After Remediation:

Once the spa has been treated, it is imperative to continue to maintain the spa in accordance with state regulations and best practices for the control of Legionella.

1. Maintain free chlorine levels between 2–10ppm (ideal 4.0-5.0),
2. Bromine residuals between 3-10ppm (ideal 4.0-6.0) and
3. pH between 7.2 to 7.8 (ideal 7.4 to 7.6).
4. Test for pH and disinfectant levels at least twice per day (hourly during periods of heavy use).
5. Maintain daily and accurate records.
6. Current ANSI/NSPI recommendations include taking the spa out of service at the end of each day to per-form a shock disinfection using 10 mg/L or 10 times the combined chlorine residual for 1 to 4 hours.
7. The World Health Organization recommends replacing at least ½ of the water volume daily.
8. On a weekly basis, the spa should be drained and thoroughly scrubbed to remove the buildup of biofilm.
9. Filters should be cleaned frequently when triggered by a drop in pressure and replaced as often as necessary.

Maintenance personnel should be trained on all aspects of the safe operation of spas and record keeping.

For more information on legionellosis visit http://www.cdc.gov/legionella/index.html

Contact Information:

County ________________________________

Contact Person __________________________

Phone Number___________________________

You may also reach the Florida Department of Health, Bureau of Epidemiology at 850-245-4401.
VII. Legionellosis Investigation Resources


http://aem.asm.org/cgi/content/abstract/72/4/2801 (abstract)  
http://aem.asm.org/cgi/reprint/72/4/2801 (full article reprint)

American Society of Heating, Refrigeration & Air-Conditioning Engineers  
http://resourcecenter.ashrae.org/store/ashrae/newstore.cgi?itemid=6916&view=item&categoryid=310&categoryparent=310&page=1&loginid=33194 (ordering information only) or  

Association of Water Technologies  
Legionella 2003: An Update and Statement by the AWT  
http://www.awt.org/IndustryResources/Legionella03.pdf

ASTM International  
D5952-02 Standard Guide for Inspecting Water Systems for Legionellae & Investigating Possible Outbreaks of Legionellosis (Legionnaires’ Disease or Pontiac Fever)  
http://www.astm.org/cgi-bin/SoftCart.exe/DATABASE.CART/REDLINE_PAGES/D5952.htm?L+mystore+asmr0805 (ordering information)

Clinical Microbiology Reviews, July 2002, pp. 506-526, Vol. 15, No. 3 Legionella and Legionnaires’ Disease: 25 Years of Investigation, Fields, Barry S.  
http://cmr.asm.org/cgi/content/full/15/3/506?view=full&pmid=12097254

European Working Group for Legionella Infections  
European Guidelines for Control and Prevention of Travel Associated Legionnaires' Disease  
http://ewgli.org/data/european_guidelines.htm

Florida Department of Health  
Legionellosis information  
http://www.doh.state.fl.us/disease_ctrl/epi/htopics/popups/leg.htm  
Guidelines for Submitting Water for Bacteriological Testing:  
http://www.doh.state.fl.us/lab/PDF_Files/GUIDELINES_WATER_BACTERIOLOGICAL.pdf

Infectious Diseases Society of America / American Thoracic Society  
http://www.journals.uchicago.edu/doi/pdf/10.1086/511159?cookieSet=1
Texas Department of State Health Services
Legionnaires' Disease in Texas (overview)
http://www.dshs.state.tx.us/idcu/disease/legionnaires/overview/

Report of the Texas Legionnaires' Disease Task Force
http://www.dshs.state.tx.us/idcu/disease/legionnaires/taskforce/

US DHHS, CDC
Legionellosis Resource Site
http://www.cdc.gov/legionella/index.htm

Procedures for the Recovery of Legionella from the Environment:

Procedures for collecting and processing environmental specimens for Legionella spp
http://www.cdc.gov/legionella/procedures.htm

Legionellosis: Legionnaire's Disease (LD) and Pontiac Fever
(general information)
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/legionellosis_q.htm
(technical information)
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/legionellosis_t.htm
(additional information)
http://www.cdc.gov/ncidod/dbmd/diseaseinfo/legionellosis_a.htm

Guidelines for Environmental Infection Control in Health Care Facilities,
MMWR Recommendations and Reports June 6, 2003/52(RR10):1-42 See Box 1 and Box 2 for
Legionellosis related guidance http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5210a1.htm
http://www.cdc.gov/mmwr/PDF/rr/rr5210.pdf

Appendix: Water Sampling Strategies and Culture Techniques for Detecting Legionellae:
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5210a2.htm

US DOL, OSHA
Safety and Health Topics - Legionnaires' Disease

Legionnaires' Disease - e-tool

OSHA Technical Manual, Chapter 7 - Legionnaires' Disease
http://www.osha.gov/dts/osta/otm/otm_iii/otm_iii_7.html

US EPA
Legionella Drinking Water Fact Sheet:
http://www.epa.gov/waterscience/criteria/humanhealth/microbial/legionellafs.pdf

Legionella Human Health Criteria Document:
http://www.epa.gov/waterscience/criteria/humanhealth/microbial/legionella.pdf

WHO
Legionella and the Prevention of Legionellosis:
http://www.who.int/water_sanitation_health/emerging/legionella.pdf
VIII. Regulatory Statutes and Authority

1. Routine Inspections/Prevention – It is suggested that, as precautionary measures, in order to prevent Legionellosis from occurring, “problem” spas and pools be closely monitored and required to correct any deficiencies in operation and sanitation. This can include administrative fines and closure.

2. Outbreaks – DOH investigators should work closely with the agency of jurisdiction during outbreak investigations and keep such agencies informed of investigation activities and progress. While most outbreaks will not require closure of an establishment, if evaluations determine that the establishment or its water facilities be closed, always begin by offering the owner/operator the opportunity to voluntarily close. If voluntary closure is not acceptable to the owner/operator, proceed with the agency of jurisdiction regarding closure.

3. DOH rule and statutory authorities
   a. s. 381.0031, F.S. - Report of diseases of public health significance to department.--
      http://www.flsenate.gov/statutes/index.cfm?App_mode=Display_Statute&Search_String=&URL=Ch0381/SEC0031.HTM&Title=->2007->Ch0381-
      >Section%200031#0381.0031
   b. s. 381.006, F.S. - Environmental health. --
      http://www.flsenate.gov/statutes/index.cfm?App_mode=Display_Statute&Search_String=&URL=Ch0381/SEC006.HTM&Title=->2007->Ch0381-
      >Section%20006#0381.006
   c. s. 381.0061, F.S. - Administrative fines.--
      http://www.flsenate.gov/statutes/index.cfm?App_mode=Display_Statute&Search_String=&URL=Ch0381/SEC0061.HTM&Title=->2007->Ch0381-
      >Section%200061#0381.0061
   d. s. 509.035, F.S. - Immediate closure due to severe public health threat. (1)(a) --
      http://www.flsenate.gov/statutes/index.cfm?App_mode=Display_Statute&Search_String=&URL=Ch0509/SEC035.HTM&Title=->2007->Ch0509-
      Section%20035#0509.035
   e. s. 64D-3.005, F.A.C. - Authority, DOH County Health Department Director or Administrator and State Health Officer.
      https://www.frrules.org/gateway/readFile.asp?sid=0&tid=2518331&type=1&file=64D-3.005.doc
   f. s. 64D-3.037, F.A.C. - Authority of the DOH County Health Department Director or Administrator and State Health Officer. ---
      https://www.frrules.org/gateway/readFile.asp?sid=0&tid=3176573&type=1&file=64D-3.037.doc
   g. s. 64D-3.041, F.A.C. - Epidemiological Investigations. --
      https://www.frrules.org/gateway/readFile.asp?sid=0&tid=3177155&type=1&file=64D-3.041.doc
Appendix A